

## New Cause for False-Positive Results with the Cryptococcal Antigen Test by Latex Agglutination

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**The highly specific and sensitive latex agglutination test for cryptococcal antigen detection in cerebrospinal fluid is routine in many hospitals. Contamination of cerebrospinal fluid by a minute amount of syneresis fluid (surface condensation) from agar gave a strongly positive reaction which was heat stable, was not eliminated by pronase treatment, and was not detected by the normal rabbit globulin controls. These observations were valid for three commercially available test kits and could represent a preventable cause of some unexplained false-positive tests despite the use of adequate controls.**

The detection of cryptococcal polysaccharide antigen in the cerebrospinal fluid (CSF) or serum by latex agglutination (LA) was first described by Bloomfield et al. (3) in 1963 and has become essential in the diagnosis and management of patients with cryptococcal disease. It is by far the most valuable fungal serodiagnostic study in clinical practice and has become a routine CSF study in many hospitals. Some patients have received treatment with amphotericin B based on the presence of cryptococcal antigen alone (7, 14). The test is reported to have a sensitivity of approximately 90% and to be nearly 100% specific when specimens are boiled and proper controls for nonspecific agglutination are performed (1, 4, 5, 8, 10). However, a small number of unexplained false-positive tests persist. We discovered that CSF, when contaminated with a minute amount of syneresis fluid (surface condensation) from a chocolate agar slant as a result of improper technique, gave a strongly positive test by LA. This positive test was neither eliminated by heating to 100°C or by pronase treatment nor detected by the normal rabbit globulin control. Three commercially available test kits gave identical results. In many hospitals the cryptococcal antigen test is performed by the microbiology laboratory, and a sample of CSF (or its supernatant) is divided into aliquots for use in the antigen determination before culturing. Failure to adhere to this sequence, or any event that results in planting of the specimen for culture before taking the sample for LA, could result in cross-contamination.

In October 1983 an 87-year-old man was admitted to our hospital for evaluation of chronic dementia. He was afebrile and had no evidence of meningitis. A lumbar puncture yielded clear CSF with normal chemistries and cell counts. The cryptococcal antigen by LA was positive to a 1:128 dilution, and the culture of CSF was negative. In a follow-up CSF sample taken a few days later, no cryptococcal antigen was detected. The patient had no evidence of malignancy, chronic meningitis, or collagen vascular disease (1, 11). A review of the laboratory procedures followed by the technologist revealed that the specimen was planted for culture before removal of a sample for LA. The capillary pipette used for inoculation of the chocolate agar slant had touched the syneresis fluid and was reintroduced into the CSF. The remainder of the same specimen was tested the next day for cryptococcal antigen and gave a false-positive test result.

After this discovery, we reviewed the records of our serology and mycology sections and studied other commercially available test kits and the constituents of chocolate agar which could have accounted for the strongly positive reaction. In the 48 months preceding our index case, 1,113 CSF samples were tested for cryptococcal antigen by our laboratory. There were 17 positive test results from nine patients. Of these nine patients, six had a positive culture for *Cryptococcus neoformans*. The remaining three patients did not, and follow-up CSF studies of each one failed to reveal cryptococcal antigen. The height of the initial titer did not differentiate true- from false-positive results (Table 1). There were no false-negative cryptococcal antigen determinations as judged by at least one culture during the same time period. All CSF specimens were tested with reagents from either International Biological Laboratories Inc. (IBL) (Cranbury, N.J.) or Meridian Diagnostics Inc. (Cincinnati, Ohio), using standard methods of testing (12), which included (i) heating to 100°C and (ii) the use of latex sensitized with rabbit anti-*C. neoformans* globulin and latex sensitized with normal rabbit globulin.

A test of the constituents of the chocolate agar syneresis fluid contained in the IBL kit (lot 3D-7237A) currently in use revealed that 50- $\mu$ l samples of syneresis fluid from GC agar base (GIBCO Laboratories, Grand Island, N.Y.), 1.0% agar, and 1.0% agarose all gave strongly positive reactions. Samples of CVA enrichment solution (GIBCO) and a 0.1% cornstarch solution did not react. We then repeated this test with 50- $\mu$ l samples of syneresis fluid from 1.0% agar, using kits from IBL (lot 34-7250), M. A. Bioproducts (Walkerville, Md.) (lot K4023), and Meridian Diagnostics (lot K4022). The samples from all three kits had positive test results in dilutions of the 1% agar syneresis fluid ranging from  $10^{-1}$  to  $10^{-3}$ . A 10- $\mu$ l sample of syneresis fluid added to 1 ml of CSF reacted positively as well. The positive reaction was not eliminated by treatment of the sample with pronase according to the method of Stockman and Roberts (15). Similar tests performed on syneresis fluid, using LA reagents for *Haemophilus influenzae*, *Neisseria meningitidis* groups A, B, and C, and *Streptococcus pneumoniae* (Directigen; Hynson, Westcott & Dunning, Baltimore, Md.), were negative.

Agar and its sulfate-free fraction agarose are acidic polysaccharides with galactose as the primary sugar (9). Since galactose makes up a small fraction (7%) of the

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TABLE 1. CSF studies of patients with positive LA tests

Patient no.	Initial cryptococcal antigen titer (no. of positive CSF samples)	CSF culture for <i>C. neoformans</i>
1	64 (1)	+
2	8 (2)	+
3	512 (4)	+
4	8 (2)	+
5	2,048 (3)	+
6	64 (2)	+
7	512 (1)	—
8	512 (1)	—
9 <sup>a</sup>	128 (1)	—

<sup>a</sup> Patient 9 was our index case.

complex polysaccharide of the cryptococcal cell wall (2, 13), we attempted to ascertain whether galactose in the syneresis fluid was responsible for the observed cross-reaction by performing an inhibition experiment with D-galactose, D-glucose, and L-galactose. Incubation of the latex reagents from the IBL kit with each sugar failed to inhibit agglutination of the latex reagent in the presence of the positive control antigen.

Since the introduction of cryptococcal antigen determination by LA, numerous studies and clinical experience have repeatedly confirmed the promising speculations about sensitivity and specificity which were made when the test was originally described. A number of commercial kits are available, and LA has replaced the more cumbersome complement fixation technique. Gordon and Vedder (8) used the slide LA and found that, of 1,003 CSF specimens tested, only 10 were positive after boiling of all specimens. Two were unexplained false-positives. Bennett and Bailey (1) demonstrated that patients with rheumatoid arthritis had positive LA tests in the absence of cryptococcal disease and that of 252 specimens (including 134 sera and 110 CSF) 24 were false-positive. They introduced the latex coated with normal rabbit globulin as a control to eliminate most non-specific agglutinations. Dolan (5) found that 3 of 20 heat-inactivated CSF samples had false-positive results, none with rheumatoid factor present. Our experience was similar. Of 1,113 CSF specimens, 17 were positive; of these, 3 were false-positive. The overall sensitivity of the LA test on CSF was 100%, and the specificity was 99.7%. These percentages are somewhat deceiving since the incidence of cryptococcal meningitis is low and since the use of the test is routine on most CSF specimens. The predictive value of a positive result was 82% and that of a negative result was 100%.

The presence of malignancy (11), chronic meningitis, or collagen vascular disease (1) is often invoked to explain the false-positive results. None of our patients had those disorders. Although we were only able to prove that contamination with agar was the cause of our index case, we feel confident that the same event occurred in at least one of the other two patients. The cross-reactivity may be due to contamination with agar of the cryptococcal antigen preparation used to immunize rabbits for commercial antisera, as was seen in the preparation of streptococcal antiserum (6),

or may have biological significance. We were unable to show that galactose was the cross-reacting antigen.

The observation that contamination of CSF with a minute amount of agar syneresis fluid causes false-positive cryptococcal antigen determinations by LA may explain at least in part the small but persistent number of false-positive test results reported. Awareness of this preventable cause of false-positive test results, combined with heat inactivation, pronase treatment, or both and with the use of controls for nonspecific agglutination, will allow the LA test for cryptococcal antigen detection in CSF to continue to be an excellent diagnostic study.

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